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Short peptides derived from the BAG-1 C-terminus inhibit the interaction between BAG-1 and HSC70 and decrease breast cancer cell growth

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ABSTRACT

BAG-1, a multifunctional protein, interacts with a plethora of cellular targets where the interaction with HSC70 and HSP70, is considered vital. Structural studies have demonstrated the C-terminal of BAG-1 forms a bundle of three alpha-helices of which helices 2 and 3 are directly involved in binding to the chaperones. Here we found peptides derived from helices 2 and 3 of BAG-1 interfered with BAG-1:HSC70 binding. We confirmed that a 12 amino-acid peptide from helix 2 directly interacted with HSC70 and when introduced into MCF-7 and ZR-75-1 cells, these peptides inhibited their growth. In conclusion, we have identified a small domain within BAG-1 which appears to play a critical role in the interaction with HSC70.

Structured summary:
MINT-7265269, MINT-7265296, MINT-7265324, MINT-7265339, MINT-7265351, MINT-7265364, MINT-7265483, MINT-7265464, MINT-7265310: *HSC70* (uniprotkb:P11142) *binds* (MI:0407) to *BAG1* (uniprotkb:Q99933) by *peptide array* (MI:0081)
MINT-7265281: *peptide 15L* (uniprotkb:Q99933) *binds* (MI:0407) to *HSC70* (uniprotkb:P11142) by *surface plasmon resonance* (MI:0107)

1. Introduction

BAG-1 is a multifunctional protein that can modulate a wide range of cellular processes [1]. For example, overexpression of BAG-1 protects cells from a wide range of apoptotic stimuli, activates RAF-1 kinase-dependent signalling, enhances proliferation and metastasis, regulates proteasomal degradation, and modulates the transcriptional activity of a variety of nuclear hormone receptors (NHR) [2–16]. Functional and expression studies suggest that overexpression of BAG-1 may play an important role in diverse cancer types [1,2,4,15,17–29].

The mechanism(s) by which BAG-1 exerts its pleiotropic effects are not fully understood. Although BAG-1 has many protein binding partners (including BCL-2, RAF-1, pRb, CHIP and the proteasome), interaction with the 70 kDa heat shock proteins, HSC70 and HSP70, is considered key for many of BAG-1 functions [5,14,28,30–33]. For example, mutations which prevent binding to HSC70/HSP70 interfere with the ability of BAG-1 to promote cell survival and to modulate NHR function [2,6,21,22,29,34–36]. In addition, BAG-1 has been shown to modulate proteasomal degradation of Tau proteins, a process dependent on the interaction with HSC70/HSP70 [5]. Moreover, BAG-1 has cochaperone activity and stimulates nucleotide exchange of HSC70/HSP70 [27,37–40] HSC70 and HSP70 play important roles in multiple cell processes, for example, via effects on protein (re)folding and activation of NHR. Thus, binding to these multifunctional proteins may explain at least in part the multiple effects associated with BAG-1 overexpression.

The interaction between BAG-1 and HSC70/HSP70 has been studied by NMR and X-ray crystallography [34,41]. The 70 kDa heat shock proteins comprise an amino terminal ATPase domain, a central peptide binding domain and a carboxyl terminal region that can form a "lid" over the peptide binding domain [42,43]. The interaction of BAG-1 with the ATPase domain of HSC70/HSP70 is mediated by the C-terminal BAG domain of BAG-1. This evolutionarily conserved "BAG domain" [44] is formed of a bundle of three alpha-helices (Fig. 1). Helices 2 and 3 are involved in elec-

Abbreviations: BAG, bcl2-associated athanogene-1; HSC70, heat shock cognate 70; HSP70, heat shock protein 70; SPR, surface plasmon resonance

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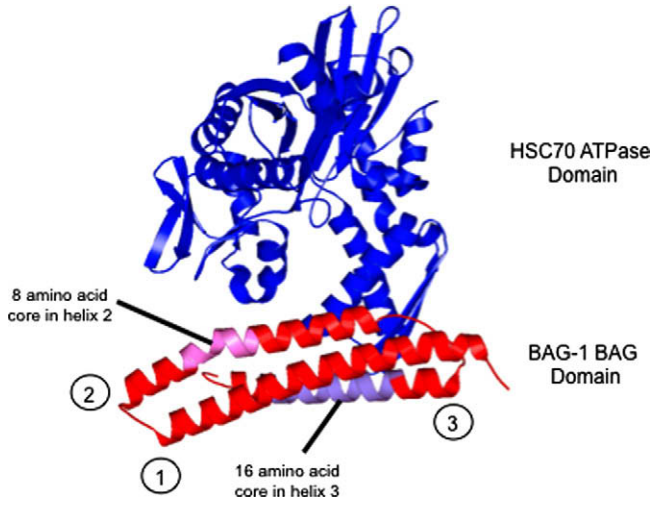

Fig. 1. The BAG-1:HSC70 interaction. The figure shows the interaction between the three helices comprising the BAG domain of BAG-1 (red) and the ATPase domain of HSC70 (blue). The 8 amino-acid binding core of helix 2 (pink) and the 16 amino-acid binding core of helix 3 (light blue) identified in this study are indicated. Figure was drawn using PyMOL© and structural coordinates from Protein Data Bank id 1hx1.

trostatic interactions with subdomains IB and IIB of the ATPase domain of HSC70 [41]. Although helix 1 is not directly involved in binding, it may contribute to intramolecular interactions which stabilize the overall structure of the BAG domain. In this paper, we have identified small peptides derived from the BAG domain that were sufficient to inhibit the interaction of BAG-1 and HSC70 and investigated their effect on breast cancer cell growth.

2. Materials and methods

2.1. BAG-1 peptides and GST-BAG-1

For in vitro studies, peptides were generated by solid phase synthesis (Mimotopes Pty Ltd., Australia), dissolved in DMSO and 10 mM stock solutions stored at −80 °C. For cell studies, Penetratin [45] and Penetratin-coupled peptides were obtained from the Cancer Research UK Research Services (London, UK), dissolved in water and 1 mM stocks stored at −80 °C. The sequence of the Penetratin-coupled peptides are shown below (Penetratin sequences are underlined).

H2-Pen CKLDRRVKATIERQIKIWFQNRRMKWKK
H2mut-Pen CKLDAAVKATIERQIKIWFQNRRMKWKK
H3-Pen LKRKRGLVKKVQAFLAECDTVERQIKIWFQNRRMKWKK
Con-Pen RQIKIWFQNRRMKWKKC-CVARTEPLQT
Penetratin RQIKIWFQNRRMKWKK

BAG-1 peptides were synthesised with Penetratin at their C-terminus coupled via a peptide bond. The control peptide was an unrelated BCL-2 derived sequence coupled to Penetratin by a disulphide bond at its N-terminus. GST and GST-BAG-1 were prepared as previously described [46], and quantified using the BioRad protein assay and bovine serum albumin (BSA) as a standard. Numbering of amino-acid residues is from the human BAG-1 sequence.

2.2. BAG-1:HSC70 interaction assay

Wells of a 96 well tissue culture plate (Griener, UK) were coated with 100 ng recombinant HSC70 (Stressgen Biotechnologies, USA) in 100 μl phosphate buffered saline (PBS) at 4 °C. The following day, wells were washed three times in PBS and non-specific binding sites were blocked with PBS containing 0.1% (w/v) BSA (Sigma, UK) for 1 h at room temperature. The wells were washed as before, prior to addition of GST-BAG-1S or GST (each 500 ng) in PBS. Following incubation at room temperature for 1.5 h, unbound protein was removed by washing in PBS. Bound BAG-1 was detected using the BAG-1-specific monoclonal antibody 3.10 G3E2 [46] at a 1/1000 dilution of ascities in PBS (200 μl). Following incubation at room temperature for 1 h, wells were washed and then incubated for 1 h with 200 μl of horseradish peroxidase conjugated sheep anti-mouse Ig antibody (GE/Amersham Biosciences, UK) diluted in PBS (200 μl). Wells were washed and developed by addition of 200 μl of o-Phenylenediamine Dihydrochloride (Sigma, UK) and incubation for 10 min at 37 °C. Absorbance at 450 nm was determined on a Dynatech MR5000 plate reader (Dynatech, Germany). The absorbance of wells containing HSC70 and GST was used to determine the 0% interaction and the absorbance of wells containing HSC70 and GST-BAG-1S was used to determine the 100% interaction. Peptides were added to the interaction assay at various concentrations prior to addition of GST-BAG-S.

2.3. Surface plasmon resonance (SPR)

SPR analysis was performed using BIAcore 2000 biosensor and BIA evaluation 2.0 software (BIAcore, Sweden). CM5 carboxymethyl dextran sensor chips were activated with injection of 100 μl of a 1:1 mixture of *N*-ethyl-*N*′-[(dimethylamino)propyl]carbodiimide and *N*-hydroxysuccinimide. Immediately after activation, 30 μl of peptide 15L (100 μM) in HBS buffer (10 nM HEPES with 0.15 M NaCl, 3.4 mM EDTA, and 0.005% surfactant P20; BIAcore, Sweden) was injected for covalent coupling to the surface of the sensor chip. Unreacted sites were blocked by injection of 70 μl of 1 M ethanolamine pH 8.5. Recombinant HSC70 was diluted to the desired concentrations in HBS buffer and injected separately over the chip-bound peptide 15L. Sensograms were collected at 25 °C, with a flow rate of 10 μl/min and data collection rate of 1 Hz. Sensograms were normalised to a base line of 0 resonance units (RU). Equivalent concentrations of each protein were injected over an untreated surface to serve as blank sensograms for subtraction of bulk refractive index background. The sensor chip surface was regenerated between runs with a 1 min injection of 100 μM HCl, at 10 μl/min.

2.4. Cell culture

ZR-75-1 and MCF-7 human breast cancer cell lines were obtained from ATCC (American Type Culture Collection, USA) and maintained in Dulbecco's modified Eagle's media (Life Technologies Inc., UK) supplemented with 10% (v/v) fetal calf serum (PAA Laboratories, UK) and 1% (v/v) penicillin/streptomycin/glutamine. To measure growth inhibition, cells were plated at a density of 5000 cells per well of a 96 well plate. The following day, cells were treated with various concentrations of BAG-1 or control peptides. After 6 days, media was removed from each well and replaced with 200 μl RPMI-1640 media (Life Technologies Inc., UK) supplemented with 10% (v/v) fetal calf serum and 1% (v/v) penicillin/streptomycin/glutamine. CellTiter 96® AQ_{ueous} One Solution Reagent (20 μl; Promega, UK) was added to the wells and the plate incubated at 37 °C for 1–4 h. Absorbance at 490 nm was measured using a Dynatech MR 5000 plate reader.

3. Results

3.1. Development of the in vitro BAG-1:HSC70 interaction assay

To investigate the ability of short peptides to disrupt the interaction between recombinant HSC70 and BAG-1S an in vitro inter-

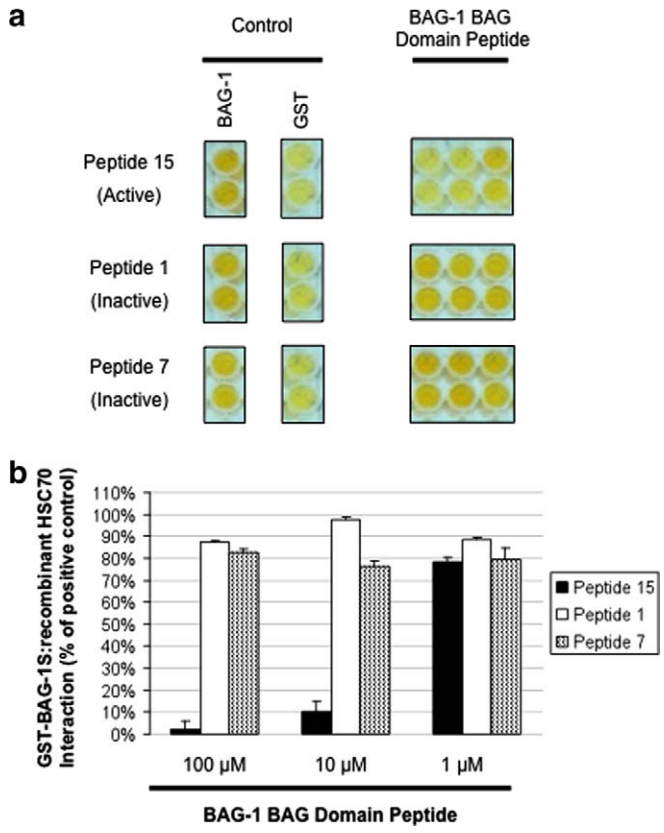

Fig. 2. Development of the in vitro BAG-1:HSC70 interaction assay. The figure shows the colorimetric reaction of the in vitro interaction between BAG-1 and recombinant HSC70. The effects of varying concentrations of BAG-1 peptides are shown in (a). Colorimetric reaction were selected peptides was measured and normalized to controls (b).

action assay was developed. Briefly, the positive control was the BAG-1 molecule interacting with HSC70 and assessed colorimetrically. GST alone was used as a negative control (Fig. 2a). The effects of varying concentrations of BAG-1 peptides on the BAG-1:HSC70 interaction was assessed (Fig. 2a). Levels of inhibition were measured by a colorimetric response and normalised to controls (Fig. 2b).

3.2. Scanning peptides

To identify minimal domains of BAG-1 sufficient to interact with HSC70, we analysed the ability of a series of 29 overlapping peptides derived from the BAG-1 C-terminus to disrupt the interaction between HSC70 and BAG-1S. The peptides were all 20 amino-acid residues in length and overlapped by 16 residues, except the final peptide which overlapped by 18 residues (Fig. 3). Peptides derived from helix 1 of the BAG domain had no significant effect on the interaction of BAG-1 and HSC70 (Fig. 4). Although peptides from helix 2 and 3 generally decreased the interaction to some extent, 6 peptides reproducibly inhibited interaction by >50% at 100 μM (peptides 12-15 from helix 2 and peptides 23 and 24 from helix 3). These results are consistent with the known role of helices 2 and 3 in mediating interaction with HSC70 [34,47]. However, since 4 contiguous peptides from helix 2 interfered with BAG-1:HSC70 binding, these data suggest that a very small region comprising ~8 amino-acid residues from helix 2 may be sufficient to bind HSC70. For helix 3, a larger region may be required to bind, since only two overlapping peptides, sharing 16 residues, were identified as strong inhibitors of binding. Dose response experiments were performed to determine EC_{50} values for some of these peptides. The EC_{50} values for peptides 13 and 15 from helix 2 were 37 ± 19 μM and 25 ± 5 μM, respectively, and the EC_{50} value values for peptides 23 and 24 from helix 3 were 30 ± 10 and 44 ± 5 μM, respectively (all mean of two determinations ± S.D.).

3.3. Mutation analysis of helix 2 and 3 peptides

We generated a second set of peptides to more precisely define regions required for inhibition of binding. Peptide 15 was selected for this study, since the putative 8 residue "core" was located at the N-terminus of this peptide (Fig. 5). Resynthesised peptide 15 (peptide 15A) was less active than the original peptide, however, this is likely to reflect differences in yield and purity of different preparations of peptides. Any deletions from the N-terminus resulted in a significant reduction in inhibitory activity (i.e. <50% inhibition at 100 μM). By contrast, deletions from the C-terminus were well tolerated until they approached the 8 amino-acid residue "core" region predicted from overlapping peptides (in peptide 15N). Consistent with this, alanine substitutions were well tolerated within the C-terminal half of the peptide, whereas alanine substitutions in the "core" region were poorly tolerated. However, there were some exceptions, for example, peptides 15Q and 15R retained good inhibitory activity.

Similar deletion and substitution analysis was performed using peptide 24 from helix 3 (Fig. 6). In this experiment, inhibition of binding at a single concentration was analysed. Similar to peptide 15, any truncations from the N-terminus inactivated the peptide, whereas C-terminal truncations were tolerated until a significant portion of the predicted core was lost (in 24I). Alanine substitutions in the C-terminal portion of the peptide were tolerated (24Q–T), whereas the effects of alanine substitutions in the N-terminal were more variable. Alanine substitution of R237 and K238 in peptide 24K significantly decreased the activity of the peptide, whereas other alanine substitutions, such as Q245 in peptide 24O were tolerated.

3.4. Surface plasmon resonance (SPR)

Our data suggest that a relatively small peptide derived from helix 2 is sufficient to bind HSC70 and prevent interaction of intact BAG-1 and HSC70. We performed SPR analysis to confirm that helix 2 peptide interacted directly with HSC70. Peptide 15L was bound to the sensor chip and addition of recombinant HSC70 to the flow channel led to a concentration dependent increase in the relative response. Removal of HSC70 from the buffer was accompanied by a return to the base line showing that the interaction between the peptide and HSC70 was fully reversible (Fig. 7). These data demonstrate that peptide 15L interacted directly with HSC70. Although we have not directly measured the kinetics of binding, the binding and dissociation of HSC70 were relatively rapid, consistent with the kinetics of the interaction of intact BAG-1 and HSC70 [48,49].

3.5. Effect of peptides on breast cancer cell growth

We determined the effects of the peptides on the growth of ZR-75-1 and MCF-7 human breast cancer cell lines (Table 1). Since peptides are poorly cell permeable, the peptides were coupled to Penetratin, a cell permeable fragment of the Antennaepaedia protein that can act as a transporter to enhance cellular uptake [45]. Peptides H2-Pen (helix 2) and H3-Pen (helix 3) inhibited the growth of breast cancer cells with EC_{50} concentrations of 25–40 μM (Table 1). By contrast, control peptides (Penetratin peptide alone or an unrelated peptide coupled to Penetratin) had no effect on cell growth (EC_{50} > 100 μM). We also analysed the growth

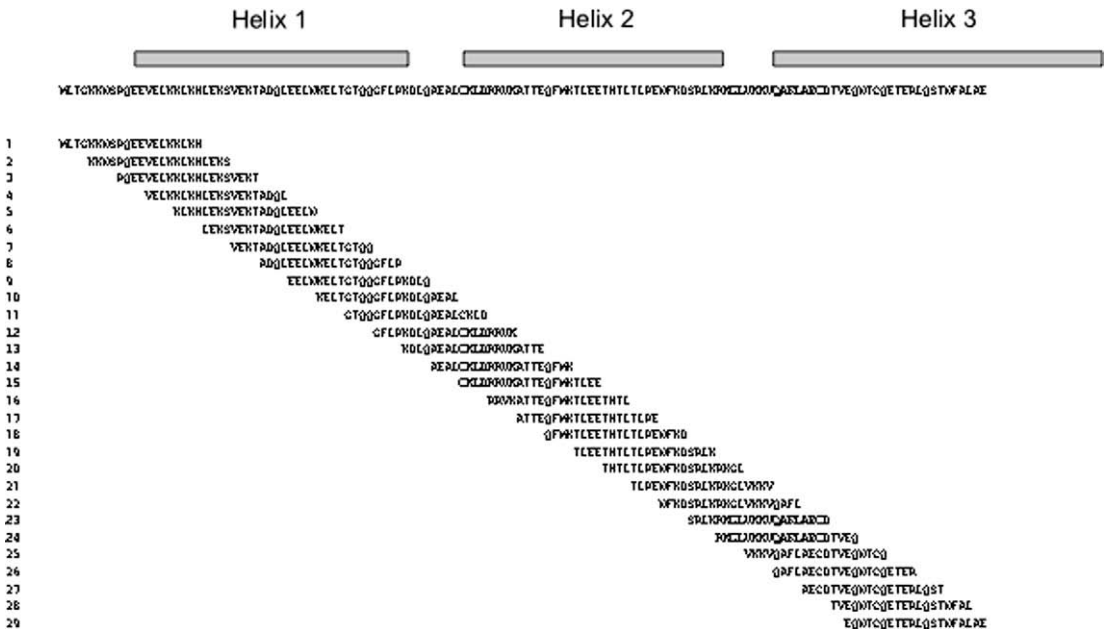

Fig. 3. Overlapping peptides used to probe the BAG-1:HSC70 interaction. The top line shows the primary amino-acid sequence and the boundaries of the three alpha-helices that comprise the BAG-1 C-terminal BAG domain. Below is shown the 29 overlapping peptides derived from this sequence.

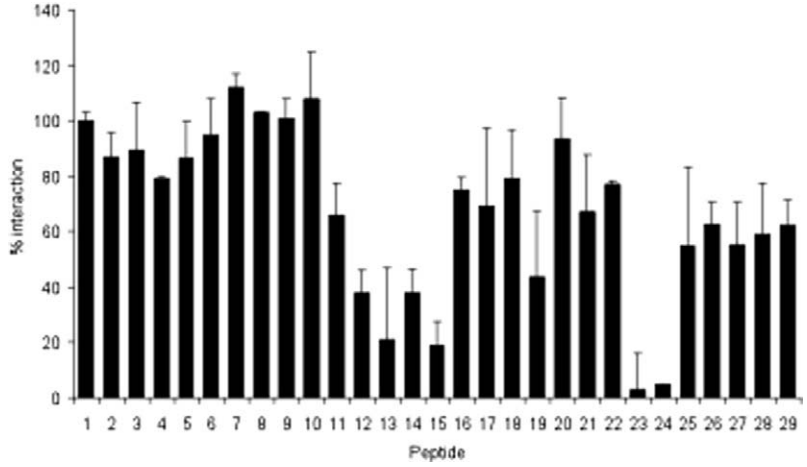

Fig. 4. Inhibition of BAG-1:HSC70 binding by 29 overlapping peptides. The 29 peptides were tested for their ability to interfere with the BAG-1:HSC70 interaction at 100 μM. The interaction of BAG-1 and HSC70 in the absence of peptide was used to set the 100% interaction level. Data shown are mean of three separate experiments each performed in duplicate ± S.D.

inhibitory activity of a mutant helix 2 peptide (H2mut-Pen) containing alanine substitutions at R205 and R206 which reduced the ability of peptide 15 to interfere with BAG-1:HSC70 binding (Fig. 5). H2mut-Pen inhibited cell growth with an EC_{50} of ~55 μM and was therefore statistically significantly less active than peptide H2-Pen ($P < 0.05$, Student's *t*-test using 6 individual samples).

4. Discussion

The interaction of BAG-1 with HSC70 and HSP70 is thought to be important for many of the functions of BAG-1 and may contribute to the activity of BAG-1 in malignant cells [1,50]. In this work, we have identified small peptides derived from helices 2 and 3 of the BAG-1 C-terminal BAG domain that prevent interaction of the intact BAG-1 protein with HSC70. For helix 2, a relatively small peptide of approx. ten amino-acid residues was sufficient to bind HSC70. Therefore, although the overall size of the C-terminal domain of BAG-1 is ~120 amino-acid residues, much smaller regions from isolated alpha-helical regions are sufficient to bind HSC70. The two minimal binding domains identified in helix 2 and 3 are juxtaposed to the HSC70 ATPase domain (Fig. 1). Peptides from helix 1 had no inhibitory activity, consistent with the fact that this helix is not directly involved in the interaction with HSC70.

NMR and X-ray crystallography studies have identified 13 amino-acid residues in the BAG domain that are thought to be involved in electrostatic interactions with the ATPase domain of HSC70 [34,41]. Seven of these amino-acid residues are in helix 2 and 4 are in helix 3, and 2 are in the loop between helix 2 and 3 (Fig. 8). Of the residues within helix 2, only R205 is contained in the minimal binding domain from this helix, suggesting that R205 plays a particularly important role in the interaction. However, it is likely that additional interactions are required for binding of this peptide to HSC70, since some other alanine substitutions

15A	**CKLDRRVK**ATIEQFMKILEE	42±7
15B	KLDRRVKATIEQFMKILEE	>100
15C	LDRRVKATIEQFMKILEE	>100
15D	DRRVKATIEQFMKILEE	>100
15E	RRVKATIEQFMKILEE	>100
15F	RVKATIEQFMKILEE	>100
15G	VKATIEQFMKILEE	>100
15H	ATIEQFMKILEE	>100
15I	IEQFMKILEE	>100
15J	CKLDRRVKATIEQFMK	20±4
15K	CKLDRRVKATIEQF	45±8
15L	CKLDRRVKATIE	6±4
15M	CKLDRRVKAT	21±8
15N	CKLDRRVK	>100
15O	**A**KLDRRVKATIEQFMKILEE	>100
15P	C**A**LDRRVKATIEQFMKILEE	>100
15Q	CK**A**DRRVKATIEQFMKILEE	20±15
15R	CKL**A**RRVKATIEQFMKILEE	9±6
15S	CKLD**A**RVKATIEQFMKILEE	>100
15T	CKLDR**A**VKATIEQFMKILEE	>100
15U	CKLDRR**AA**ATIEQFMKILEE	>100
15V	CKLDRRVKA**A**IEQFMKILEE	7±5
15W	CKLDRRVKAT**AA**QFMKILEE	10±4
15X	CKLDRRVKATIE**AA**MKILEE	21±8
15Y	CKLDRRVKATIEQF**AA**ILEE	>100
15Z	CKLDRRVKATIEQFMK**AA**EE	15±7
15AA	CKLDRRVKATIEQFMKIL**AA**	10±3

Fig. 5. Mutational analysis of helix 2 peptide 15. The figure shows the sequence of peptides derived from peptide 15, and EC_{50} values for inhibition of the BAG-1:HSC70 interaction. Alanine substitutions are shown in bold and the predicted "core" sequences are indicated. Data are derived from a minimum of two (up to four) separate experiments.

24A	**RKGLVKKVQAFLAECD**TVEQ	8±2%
24B	GLVKKVQAFLAECDTVEQ	76±26%
24C	VKKVQAFLAECDTVEQ	85±12%
24D	KVQAFLAECDTVEQ	97±6%
24E	QAFLAECDTVEQ	114±22%
24F	FLAECDTVEQ	109±3%
24G	RKGLVKKVQAFLAECD	23±2%
24H	RKGLVKKVQAFLAE	3±12%
24I	RKGLVKKVQAFL	70±25%
24J	RKGLVKKVQA	40±12%
24K	**AA**GLVKKVQAFLAECDTVEQ	78±16%
24L	RK**AA**VKKVQAFLAECDTVEQ	37±7%
24M	RKGL**AA**KVQAFLAECDTVEQ	37±8%
24N	RKGLVK**AA**QAFLAECDTVEQ	54±23%
24O	RKGLVKKV**AA**FLAECDTVEQ	22±7%
24P	RKGLVKKVQA**AA**AECDTVEQ	46±3%
24Q	RKGLVKKVQAFL**AA**CDTVEQ	1±22%
24R	RKGLVKKVQAFLAE**AA**TVEQ	33±18%
24S	RKGLVKKVQAFLAECD**AA**EQ	6±3%
24T	RKGLVKKVQAFLAECDTV**AA**	5±6%

Fig. 6. Mutational analysis of helix 3 peptide 24. The figure shows the sequence of peptides derived from peptide 24, and values for inhibition of the BAG-1:HSC70 interaction at 100 μM. Alanine substitutions are shown in bold and the predicted "core" sequences are indicated. Data are the mean interaction in the presence of peptide (±S.D.) derived from 2 experiments each performed in duplicate. The interaction of BAG-1 and HSC70 in the absence of peptide was used to set the 100% interaction level.

also inactivated the peptide. Substitution at some positions was tolerated and in general, these positions lie on the face of the alpha-helix that would be predicted to orientate away from HSC70. Overall, the data suggest that R205 represents a "hot-spot" on the interaction interface between BAG-1 and HSC70, and relatively small peptides containing this region are particularly effective at interfering with binding of intact BAG-1.

The minimal binding domain of helix 3 was larger, comprising ~16 amino-acid residues. This domain contained all four of the residues within helix 3 that were predicted to contribute electrostatic

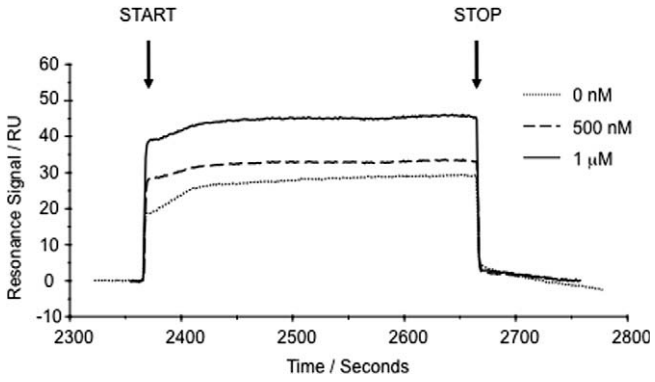

Fig. 7. SPR analysis. Peptide 15L was coupled to the BIAcore CM5 sensor chip and recombinant HSC70 introduced in to the flow channel. The graph shows the changes in resonance signal over time detected with 0 nM, 500 nM or 1 μM HSC70. Experiment shown is representative of two similar experiments.

Table 1
Growth inhibition by BAG-1 peptides in breast cancer cell lines. EC_{50} for growth inhibition (μM) is shown. Values are mean ± S.E. of the mean of three separate experiments performed in duplicate.

Peptide	ZR-75-1	MCF-7
H3-Pen	39.1 ± 3.6	37.2 ± 1.7
H2-Pen	26.7 ± 2.1*	39.9 ± 1.8*
H2mut-Pen	53.2 ± 3.0*	56.9 ± 0.7*
Con-Pen	>100	>100
Penetratin	>100	>100

* $P < 0.05$.

interactions from structural studies [41] (Fig. 8). Overall, truncations and alanine substitutions of peptide 24 were more variable. This suggests that multiple residues contribute significant free energy to the interaction of BAG-1 helix 3 with HSC70.

Although the importance of HSC70/HSP70 binding for the function of overexpressed BAG-1 is well described, the significance of these interactions for endogenous BAG-1 is not known. We coupled helix 2 and 3 derived peptides to Penetratin to facilitate cellular uptake and analysed their effects on cell growth of breast cancer cells. Overexpression of BAG-1 in breast cancer has been associated with outcome [21,51,52], so this is a relevant system to study BAG-1 function. Both BAG-1 derived peptides inhibited the growth of cells, whereas control peptides had no effect. A helix 2 peptide with alanine substitutions at R205 and R206 was significantly less active, consistent with these peptides acting via BAG-1/HSC70. However, this peptide did retain some growth inhibitory activity. This may relate to some retained affinity for HSC70, or possibly non-specific cytotoxic effects that have been observed previously with alpha-helical peptides [53].

We postulate that these small peptides derived from the BAG domain of BAG-1 exhibit their cellular effects by binding the ATPase domain of HSC70 thus preventing the interaction between BAG-1 and HSC70 (Fig. 9). BAG-1 has been shown to bind many of its cellular targets via HSC70 and therefore disrupting this interaction may lead to a loss of BAG-1 function in breast cancer cells.

Moreover, we have recently shown that small molecules which disrupt BAG-1 C-terminal protein:protein interactions inhibit the growth of breast cancer cells and diminish BAG-1 function in cells [54]. In the current study, we have interrogated the BAG-1 C-terminal further and have demonstrated that short peptides derived from the BAG domain of BAG-1 bind to the ATPase domain of HSC70 and prevent the direct binding of BAG-1. This interaction is critical for a variety of the functions attributed to BAG-1 in breast

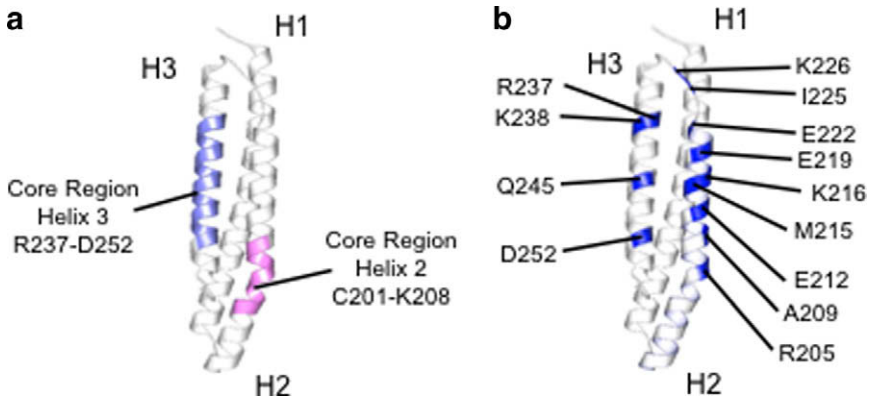

Fig. 8. Structure of the BAG-1 HSC70 binding interface. The structure of the BAG-1 C-terminal domain viewed from the position of HSC70 in the BAG-1:HSC70 complex. Figures were drawn using PyMOL© and coordinates from PDB id 1hx1. The position of the 3 alpha helices are shown. (a) The position of the minimal binding domains is indicated. (b) All BAG residues predicted to be involved in electrostatic interactions with HSC70 are shown in blue [41].

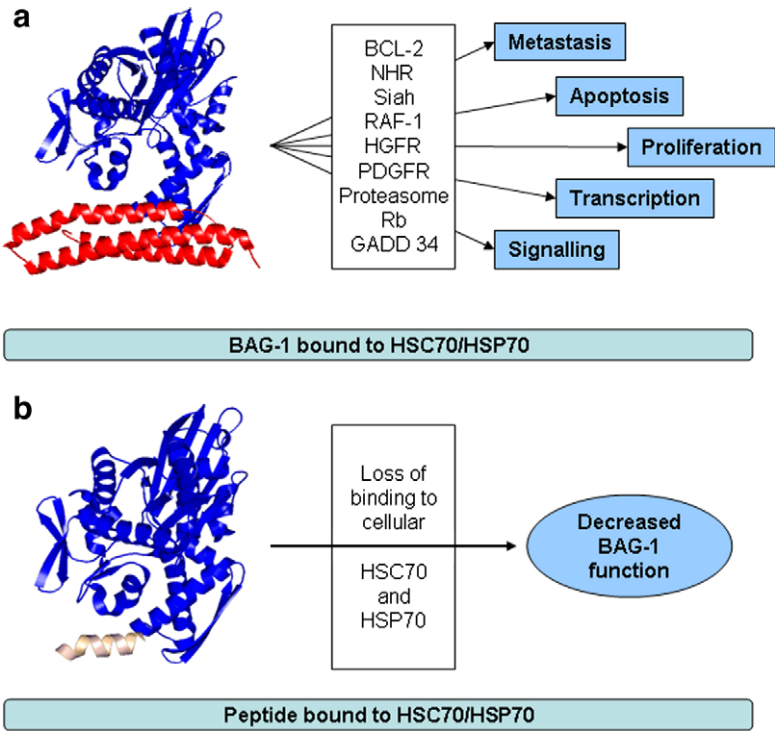

Fig. 9. Potential mechanisms for the biological effects of BAG-1 peptides. BAG-1 is a multifunctional protein that interacts with multiple cellular targets and modulates a wide range of cellular processes (a) [1]. The C-terminal BAG domain of BAG-1 (red) has been shown to interact with the ATPase domain (blue) of the heat shock chaperone molecule, HSC70 [34,41]. This interaction has been shown to be critical for the interaction between BAG-1 and many of its cellular targets, and may in part, explain the multiple effects associated with BAG-1 [21,29]. Small molecules that block these protein:protein interactions mediated by the BAG domain have been shown to inhibit the growth of breast cancer cells [54]. Consistent with this, we have shown that small peptides derived from the BAG domain of BAG-1 both block the interaction between BAG-1 and HSC70, and inhibit the growth of breast cancer cells. We hypothesise that small peptides (white) derived from the BAG domain of BAG-1 bind to the ATPase domain (blue) of HSC70 preventing the binding of BAG-1 (b). This in turn prevents BAG-1 interacting with many of its cellular targets for which it requires binding to the heat shock chaperone molecules. Therefore it is conceivable that small peptides that block the interaction between BAG-1 and HSC70 will decrease the activity of BAG-1 in breast cancer cell lines. Figures were drawn using PyMOL© and structural coordinates from Protein Data Bank id 1hx1.

cancer cells [21,22,29]. Consistent with this, disruption of the interaction may lead to a loss of BAG-1 function in breast cancer cells (Fig. 9).

In this current study we have identified minimal HSC70 binding domains located within the BAG-1 C-terminal domain and demonstrated that relatively small peptides are sufficient to interact with HSC70. Thus, the BAG-1:HSC70 interaction may be an attractive target for novel anti-cancer therapies aimed at inactivating this multifunctional protein. Despite the difficulty in developing drugs to target protein:protein interactions, the identification of relatively small active peptides and "hot-spots" within the BAG-1:HSC70-binding interface suggests, as demonstrated in by our recent small molecules investigations, that it may be possible to identify small chemical inhibitors of the BAG-1:HSC70 interaction [54].

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